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INCIDENCE OF SHIGA TOXINS PRODUCING *ESCHERICHIA COLI* IN MEAT, MINCED MEAT, POULTRY MEAT AND CHILDREN DIARRHEA

MOHAMED KARMI [1] and SUSAN A. ISMAIL [2]

[1] Department of Food Hygiene, Faculty of Veterinary Medicine, Aswan University, 81528 Aswan, Egypt.

[2] Department of Microbiology, Animal Health Research Institute (AHRI), Dokki.

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ABSTRACT

A total of 200 samples of meat, minced meat, poultry meat and children diarrhea were collected from different shops in Aswan during 2018. These samples consist of 50 samples of each meat, minced meat, poultry meat and children diarrhea. Bacteriological examination of the samples was carried out for detection of Shiga toxins-producing *Escherichia coli* (STEC) followed by serological and genetic characterization for serotyping and for detection of some virulence genes including *stx1*, *stx2* and *eae*A genes. The incidence of *E. coli* in meat, minced meat, poultry meat and children diarrhea was (11/50) 22%, (9/50) 18%, (6/50) 12% and (6/50) 12%, respectively. Incidence of Shigatoxin – forming genes; *stx*1 ranged from 33-77% and *stx*2 ranged from 36-83%, while *eae*A ranged from 16-36%. STEC were found in meat, minced meat, and poultry meat and transferred to children through contaminated food. Good processing procedures and sufficient temperatures should be confirmed during meat preparation to avoid infection and possible food poisoning from this dangerous pathogen.

Key words: Shiga toxins producing *E. coli*, meat, poultry meat, virulence genes.

INTRODUCTION

Shiga toxins producing *Escherichia coli* (STEC) lives in the intestines of animals especially ruminants which considered the main source of infection for humans. STEC contaminates meat during slaughter, evisceration, handling and processing and found on the carcass surface, in minced meat and in undercooked burgers (Fries *et al.*, 1996; Zhao *et al*., 2001). STEC are characterized basically by the production of Shigatoxins which causing cytotoxicity of the host cells through inhibition of protein synthesis, these toxins are not found in EPEC (Nataro and Kaper, 1998). STEC (or verotoxins producing *Escherichia coli*, VTEC) are very hazardeous group where it causing severe food poisoning; start from enteritis and ending by hemorrhagic colitis (HC), hemolytic uremic syndrome (HUS) and renal failure in human (Beutin *et al*., 2004; Blanco *et al*., 2004; Bolton, 2011). Symptoms of STEC infection start after 3-8 days and recovered after 10 days, some cases complicated to HC and about 3-7% into HUS (FSANZ, 2013). All human ages are susceptible for infection especially children and elderly (ICMSF, 2002). Normally infections start by HC and then complicated into HUS, although some atypical cases of HUS starts directly without diarrhea (Verweyen *et al.*, 2000). In addition to HC and HUS, STEC causes neonatal meningitis, nasocomial septicaemia and post surgical infections (Falagas and Gorbach, 1995). O157:H7 serotypes are more virulent than non-O157:H7 strains, although some non-O157:H7 are similar in virulence to O157:H7 causing mild non bloody diarrhea to HUS and death (Johnson *et al*., 2006). The main non-O157:H7 STEC strains are O26, O45, O103, O111, O121 and O145 and having the virulence genes of Shigatoxins and adhesion protein (*stx* and *eae*). Similarly, the most implicated foods are ground beef, burgers and cattle considered the main reservoir for non-O157:H7 strains (Arthur *et al.*, 2002; Eklund *et al*., 2002; Liao *et al*., 2014). About 400 serotypes of STEC were identified and about 100 of them were described as a main cause of severe human infections and having the ability to produce one or more Shiga toxins (Bergey's manual 2005). The similarity rate between STEC strains isolated from food and from human patients are 60%, so that Food was considered the most important and main source of infections (Miko *et al.*, 2009). Meats have a special importance in transmission of STEC infection to human being which contaminated during slaughter or preparation (Cohen *et al*., 2007). In this work, we study the incidence of STEC in meat, minced meat, poultry and children diarrhea, serotyping of the isolates as well as some virulence genes such as Shiga toxins forming genes and adhesion protein gene.

Corresponding author: Dr. Mohamed Karmi
E-mail address: karmy99@ yahoo.com
Present address: Department of Food Hygiene, Faculty of Veterinary Medicine, Aswan University, 81528 Aswan, Egypt

MATERIALS AND METHODS

Samples

A total of 200 samples of meat, minced meat and poultry meat were collected from different shops in Aswan during 2018. Sterile swabs in 10 ml BPW were used for taking diarrheal swabs which were collected from children hospitals in Aswan city. These samples consist of 50 samples of each meat, minced meat, poultry meat and children diarrhea. Samples were transferred in low temperature to the laboratory in the faculty of veterinary medicine, Aswan University for bacteriological, biochemical, serological and genetic assays of STEC.

Isolation and Identification

Twenty five grams of each meat sample were aseptically transferred to sterile stomacher bag containing 225 ml Buffered Peptone Water (BPW). The bag content was homogenized using a Stomacher® 400 Circulator (Seward Ltd., UK) for 3 minute and incubated at 37 ºC for 12 hours. Loopful (10 µl) was taken from each BPW enrichment culture and from diarrheal swab wash after 12 hours incubation at 37°C and streaked on Eosin Methylene Blue Agar (EMB) plates (Oxoid, Code: CM0069) the culture plates were incubated aerobically at 37°C for 24 hours for EMB plates. Olive green colonies with metallic sheen on EMB were positive for pathogenic *E. coli*. Positive colonies were picked up for further biochemical and serological confirmation. Positive strains were confirmed with Gram's staining, Indole production, Methyl Red, Voges-Proskauer, Simmon`s citrate, Urease production and Triple Sugar Irone agar (Quinn *et al.*, 1994; Adams and Moss, 1999). Positive E. coli isolates were tested for presence of somatic (O) and flagellar (H) antigens using latex agglutination test (Hampshire, UK) (Krishnan *et al*., 1987; March and Ratnam, 1989).

Genetic Characterization

Multiplex PCR assay of some virulence genes of *E. coli* O157:H7 including Shiga toxins-forming genes (stx1, stx2) and intimin gene (eaeA). DNA extraction was carried out by using QIAamp Mini Kit (Catalogue No: 51304) DNA purification kits (Qiagen, Germany) according to the manufacturer instructions. Primer sequences were used for stx1, stx2 and eaeA genes amplification (Gannon *et al.,* 1992, 1997) (Table 1). Multiplex PCR amplification of *stx*1, *stx*2 and *eae*A genes was carried out. Each reaction consists of 2.5 µl of 10x buffer, 12.5 µl master mix (Emerald Amp GT master mix (Takara, Code: RR310A), 1 µl each primer of 20 pmol concentrations, 6 µl DNA template and nuclease free water till 25 µl volume. Thermacycler (Eppendorf, Germany) was used with initial denaturation step at 94°C for 5 minutes followed by 30 PCR cycles, each consisting of 1 min of denaturation at 94°C; 1 minute of annealing at 58°C for 1 minute; extention at 72°C and final extension at 72°C for 10 minutes. PCR reaction mixtures were electrophoresed on 1.5% agarose gels and stained with ethidium bromide and visualized on UV transilluminator (Biometra) and analyzed using Biodoc Analyse Biomet (EL-Jakee *et al*., 2009).

RESULTS

The incidence of *E. coli* in meat, minced meat, poultry meat and children diarrhea was (11/50) 22%, (9/50) 18%, (6/50) 12% and (6/50) 12%, respectively (Table 2). *E. coli* serotypes isolated from meat were O111:H2, O125:H21, O128:H2, O26:H11, O55:H7 and O124, from minced meat were O128:H2, O119:H4, O44:H18, O26:H11, O111:H2, O55:H7, O78, from the poultry meat were O26:H11, O114:H21, O119:H4, O125:H21, O2:H6 and from children diarrhea were O119:H4, O111:H2, O128:H2, O114:H21, O124 (Table 3,4). Incidence of virulence genes were as the following; *stx*1 was (5/11) 45%, (7/9) 77%, (2/6) 33%, (3/6) 50%, stx2 was (4/11) 36%, (4/9) 44%, (5/6) 83%, (3/6) 50% and eaeA was (4/11) 36%, (3/9) 33%, (2/6) 33%, (1/6) 16% in meat, minced meat, poultry meat and children diarrhea were, respectively (Table 5).

Table 1: Primer sequences of virulence genes in *E. coli*.

Primer	Oligonucleotide Sequence (5`- 3`)	Product Size (bp)	References
*stx*1 (F)	ACA CTG GAT GAT CTC AGT GG	614	Gannon *et al.*, 1992
*stx*1 (R)	CTG AAT CCC CCT CCA TTA TG		
*stx*2 (F)	CCA TGA CAA CGG ACA GCA GTT	779	Gannon *et al.*, 1992
*stx*2 (R)	CCT GTC AAC TGA GCA GCA CTT TG		
eae A (F)	GTG GCG AAT ACT GGC GAG ACT	890	Gannon *et al.*, 1997
eae A (R)	CCC CAT TCT TTT TCA CCG TCG		

Table 2: Incidence of *E. coli* in meat, minced meat, poultry meat and children diarrhea.

Samples	Number	Positive number	Percentage
Meat	50	11	22
Minced meat	50	9	18
Poultry meat	50	6	12
Children diarrhea	50	6	12
Total	200	32	16

Table 3: Serotypes of *E. coli* in meat, minced meat, poultry meat and children diarrhea.

Samples	Serotype	Number	Percentage	*E. coli* group
Meat	O111 : H2	3	27%	EHEC
	O55 : H7	3	27%	EPEC
	O125 : H21	2	18%	ETEC
	O128 : H2	1	9 %	ETEC
	O26 : H11	1	9%	EHEC
	O124	1	9%	EIEC
Minced meat	O128 : H2	3	33%	ETEC
	O119 : H4	1	11%	EPEC
	O44 : H18	1	11%	EPEC
	O26 : H11	1	11%	EHEC
	O111 : H2	1	11%	EHEC
	O78	1	11%	EPEC
	O55 : H7	1	11%	EPEC
Poultry meat	O26 : H11	2	33%	EHEC
	O114 : H21	1	11%	EPEC
	O119 : H4	1	11%	EPEC
	O2 : H6	1	11%	EPEC
	O125 : H21	1	11%	ETEC
Children diarrhea	O119 : H4	1	16%	EPEC
	O111 : H2	2	33%	EHEC
	O128 : H2	1	16%	ETEC
	O124	1	16%	EIEC
	O114 : H21	1	16%	EPEC

Table 4: Serotypic and genetic characterization of *E. coli* in meat, minced meat, poultry meat and children diarrhea.

Serotype	*stx1*	*stx2*	*eaeA*	Source
O26: H11	+	+	+	Chicken& meat and minced meat
O114: H21	-	+	-	Chicken and children stool
O119: H4	+	+	-	Chicken& minced meat and children stool
O2 : H6	-	+	-	Chicken
O125: H21	-	+	+	Chicken
O111: H2	+	+	+	Meat& minced meat and children stool
O55: H7	+	-	+	Meat and minced meat
O125: H21	-	+	+	Meat
O124	-	-	-	Meat and children stool
O128: H2	+	-	-	Meat &minced meat and children stool
O44: H18	+	-	-	Minced meat
O78	+	+	-	Minced meat

Table 5: Serotypic and genetic characterization of *E. coli* in meat, minced meat, poultry meat and children diarrhea.

Sample	*stx1*		*stx2*		*eae*A	
	Number	Percentage	Number	Percentage	Number	Percentage
Meat	5	45	4	36	4	36
Minced meat	7	77	4	44	3	33
Poultry meat	2	33	5	83	2	33
Children diarrhea	3	50	3	50	1	16

DISCUSSION

In this study, results revealed that incidence of *E. coli* in meat (22%) and minced meat (18%) were higher than that in poultry meat (12%), contamination by *E. coli* in fresh meat and minced meat was higher than that of poultry meat may be due to the *E. coli* is a normal inhabitant of the large intestines of cattle and higher possibility of meat contamination during slaughter, evisceration, transport, preparation. Also, cattle are the main reservoir of STEC (Meng *et al*., 2013). Furthermore, minced meat may get contaminated from several steps during handling, mincing and storage so that it may be more harmful to the consumers and health (Eldaly *et al*., 1988). Incidence of *E. coli* in meat (22%) was lower than Momtaz *et al*. (2013) (29%), Farhan *et al*. (2014) (30%), Patricia *et al*. (2014) (36.1%) and Hyun-Jung *et al*. (2015) (42.3%) but higher than Kesava *et al*., 2011 (0.6%), Mohamed *et al*. (2013) (11%) and Sethulekshmi *et al*. (2016) (12.5%). Incidence of *E. coli* in minced meat (18%) was higher than Wenting *et al*. (2012) (5.2%), Perelle *et al*. (2007) (11%) and Mora *et al*. (2007) (11%), lower than Acheson, (2000) (21%), Panahee and Pourtaghi, (2016) (23.5%), Zaki and Elmahrouk, 2005 (44%), Badri *et al*. (2009) (45%), Hazarika *et al*. (2004) (56.8%) and Soliman and Tabiy (2006) (68%). Incidence of *E. coli* in poultry meat (12%) was similar to Momtaz *et al*., 2012 (11%), lower than Zende *et al*. (2013) (16.67%) and Nguyen *et al*. (2016) (92.7%), Hyun-Jung *et al*. (2015) (75.9%), Eyy and Arslan, 2012 (87.5%), Zhao *et al*. (2001) (38.7) and Momtaz and Jamshidi, (2012) (34.93%). Incidence in children diarrhea (12%) was similar to Elsheikh and Alassouli, (2001) (13%), lower than Bitzan *et al*. (1993) (51%), Awadallah *et al*. (2014) (20%), higher than Christina *et al*. (2011), (0.3%) and Kesava *et al*., 2011 (1.3%). Serotypes O111:H2 and O128:H2 were detected in meat, minced meat and diarrhea while serotype O119:H4 was detected in minced meat, poultry meat and diarrhea. Serotype O26:H11 was detected in meat, minced meat and poultry meat while serotype O114:H21 was detected in poultry meat and diarrhea. Serotype O125:H21 was detected in meat and poultry meat while serotype O124 was detected in meat and diarrhea. Serotype O55:H7 was detected in meat and minced meat while serotype O44:H18 and O78 were detected only in minced meat and serotype O2:H6 was detected only in poultry meat. It is noticed that most of serotypes were found in meat and minced meat and similar to serotypes of children diarrhea, there is a strong relation between serotypes of beef meat and human infections. O4, O5, O16, O26, O46, O48, O55, O91, O98, O111ab, O113, O117, O118, O119, O125, O126, O128, O145, O157 and O172 are the most famous EHEC serogroups. Beside the novel discovered serogroups such as: O176, O177, O178, O179, O180 and O181 (Scheutz and Strockbine, 2005). Identification of STEC strains depends basically on serological typing by using O and H antigens (Gyles, 2007). Not only O157 but also non-O157 strains are implicated in sever diseases to the consumers and in certain localities it may be more common than O157 in causing diarrhea and HUS (Pradel *et al*., 2000). Some authors stated that non-O157 STEC infections may be milder than O157 infections (Brooks *et al*., 2005). Shigatoxin forming genes (*stx*1, *stx*2) and adhesion gene (*eae*A) were found in about (17/32) 53% of isolated *E. coli*, most of them occur in meat and minced meat and lower in poultry meat and children diarrhea. Presence of such genes in isolates is indication for contamination of meat with highly virulent strains of *E. coli* and higher potential of sever food poisoning infection to the consumers. Meat and meat products are frequently contaminated during preparation by the workers. Production of shigatoxins and adhesion protein intimin is the most characteristic feature of STEC. Shigatoxins (stx1 and stx2) have a cytotoxic effect through inhibition of protein synthsis and cell death while the adhesion protein, intimin, is a surface protein of cell wall which facilitates attachment of the bacteria to the intestinal cells. Genes; *stx*1 and *stx*2 are found in the temperate lambdoid bacteriophages of *E. coli* chromosome while *eae*A gene is found in the pathogenicity island in the chromosome, known as the locus of enterocyte effacement (LEE) (Paton and paton, 1998).

CONCLUSION

Shigatoxin producing *E. coli* contaminate red meat, minced meat, poultry meat by relative incidence rates. Infection transferred to human being through

consumption of meat and poultry meat that manifested in positive samples of children diarrhea. These Serotypes produce dangerous Shiga toxins which responsible for food poisoning, bloody diarrhea, Hemorrhagic Colitis and Hemolytic-Uremic Syndrome. Sufficient processing temperature should be confirmed during preparation to decrease incidence of high risk food poisoning and diseases.

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REFERENCES

Acheson, D.W.K. (2000): How does *Escherichia coli* O157:H7 testing in meat compare with what we are seeing clinically? Journal of Food Protection. 63(6): 819–821.

Adams, M.R. and Moss, M.O. (1999): Food microbiology. The Royal Society of Chemistry, Thomas Graham house, Service Park, Cambridge, UK. 192-202.

Arthur, T.M.; Barkocy-Gallagher, G.A.; Rivera-Betancourt, M. and Koohmaraie, M. (2002): Prevalence and characterization of non-O157 Shiga toxin-producing *Escherichia coli* on carcasses in commercial beef cattle processing plants. Appl Environ Microbiol. 68 (10): 4847–4852.

Awadallah, M.A.I.; Ahmed, H.A. and Merwad, A.M. (2014): Prevalence of Non- O157 Shiga Toxin-Producing *Escherichia coli* and Enterotoxigenic Staphylococci in Ready-to-eat Meat Products, Handlers and Consumers in Cairo, Egypt. Global Veterinaria 12 (5): 692-699.

Badri, S.; Filliol, I.; Carle, I.; Hassar, M.; Fassouane, A. and Cohen, N. (2009): Prevalence of virulence genes in *Escherichia coli* isolated from food in Casablanca (Morocco). J. Food Control. (20):560-564.

Bergey's manual of systematic bacteriology. (2005): The Proteobacteria, Vol. 2, 2nd edition.

Beutin, L.; Steinruck, H.; Krause, G.; Steege, K.; Haby, S.; Hultsch, Beutin, L.; Krause, G.; Zimmermann, S.; Kaulfuss, S. and Gleier, K. (2004): Characterization of Shiga toxin-producing *Escherichia coli* strains isolated from human patients in Germany over a 3-year period. J. Clin. Microbiol. 42: 1099–1108.

Bitzan, M.; Ludwig, K.; Klemt, M.; Konig, H.; Buren, J. and Muller-Wiefel, D.E. (1993): The role of *Escherichia coli* O157 infections in the classical (enteropathic) haemolytic uraemic syndrome. results of a central european, multicentre study. Epidemiol Infect.110: 183–96.

Blanco, J.E.M.; Blanco, M.P.; Alonso, A.; Mora, G.; Dahbi, M.A. Coira, and Blanco, J. (2004): Serotypes, virulence genes, and intimin types of Shiga toxin (verotoxin)-producing *Escherichia coli* isolates from human patients: prevalence in Lugo, Spain, from 1992 through 1999. J. Clin. Microbiol. 42: 311– 319.

Bolton, D.J. (2011): Verocytotoxigenic (Shiga toxin-producing) *Escherichia coli*: virulence factors and pathogenicity in the farm to fork paradigm. Food borne Pathog Dis., 8(3): 357-65. doi: 10.1089/fpd.2010.0699.

Brooks, J.T.; Sowers, E.G.; Wells, J.G.; Greene, K.D.; Griffin, P.M.; Hoekstra, R.M. and Strockbine, N.A. (2005): Non-O157 Shiga toxin-producing *Escherichia coli* infections in the United States, 1983-2002. J Infect Dis. 192(8): 1422-9.

Christina, R. Hermos.; Marcie, Janineh.; Linda, L. Han. and Alexander, J. Mc. Adam. (2011): Shiga Toxin-Producing *Escherichia coli* in Children: Diagnosis and Clinical Manifestations of O157:H7 and Non-O157:H7 Infection. 49(3): 955–959.

Cohen, N.; Ennaji, H.; Bouchrif, B.; Hassar, M. and Karib, H. (2007): Comparative study of microbiological quality of raw poultry meat at various seasons and different slaughtering process in Casablanca (Morocco). J. Appl. Poultry Res. 16: 502-508.

Eklund, M.; Leino, K. and Siitonen, A. (2002): Clinical *Escherichia coli* Strains Carrying *stx* Genes: *stx* Variants and *stx*-Positive Virulence Profiles. J Clin Microbiol., 40(12): 4585–4593. doi: 10.1128/JCM.40.12.4585-4593.2002

Eldaly, E.; Ibrahim, A. and Abd Elgalil, Y. (1988): Prevalence of *Escherichia coli* in raw minced meat the bulletin of high institute of public health .18 (5): 1123-1129.

EL-Jakee, J.; Moussa, E.; Mohamed, K. and Mohamed, G. (2009): Using Molecular techniques for characterization of *Escherichia coli* isolated from water sources in Egypt. Global Veterinaria 3 (5): 354-362.

El-Sheikh, K.H. and El-Assouli, S.M. (2001): Prevalence of viral, bacterial and parasitic Enteropathogens among young children with acute diarrhea in Jeddah, Saudi Arabia. J. Health Popul. Nutr.19 (1): 25-30.

Eyy, A. and Arslan, S. (2012): Prevalence of *Escherichia coli* in retail poultry meat. ground beef and beef. Med. Weter. 68 (4): 237-240.

Falagas, M. and Gorbach, S. (1995): Practice guidelines: Urinary tract infections.

Infectious Diseases in Clinical Practice, (4), 241–257.

Farhan, R.S.; Abdalla, H.A.; Abdelrahaman, N. Fahmy. and Salama, E. (2014): Prevalence of *Escherichia Coli* in Some Selected Foods and Children Stools with Special Reference to Molecular Characterization of Enterohemorrhagic Strain. American Journal of Animal and Veterinary Sciences. 9 (4): 245- 251.

Fries, R.; Humphery, G.T.J.; Nesbakken, T.R.; Schott, W.A.W.W. and Skovgaard, N. (1996): Microbial control in the meat industry. 7. Bacterial pathogens on raw meat and their properties, p. 34 pp. University of Bristol Press, UK, Bristol.

FSANZ (2013): Agents of food borne illness. 2nd ed, Food Standards Australia New Zealand, Canberra.

Gannon, V.P.J.; King, R.K.; Kim, J.Y. and Golsteyn-Thomas, E.J. (1992): Rapid and sensitive method for detection of Shiga-like toxin-producing *Escherichia coli* in ground beef using the polymerase chain reaction. Appl. Environ. Microbiol.58: 3809–3815.

Gannon, V.P.J.; Souza, S.D.; Graham, T.; King, R.K.; Rahn, K. and Read, S. (1997): Use of the flagellar H7 gene as a target in multiplex PCR assays and improved specificity in identification of enterohemorrhagic *Escherichia coli* strains. J. Clin. Microbiol. 35: 656–662.

Gyles, C.L. (2007): Shiga toxin-producing *Escherichia coli*. Journal of Animal Science 85(13): 45-62.

Hazarika, R.A.; Singh, D.K.; Kapoor, K.N. and Agorwal, R.K. (2004): Prevalence of different serotypes of *Escherichia coli* in meat and its products. Indian J. Comb. microbiol .Infect .Dis. 25(1): 19-22.

Hyun-jung, Park.; Jang, Won. Yoon.; Eun-Jeong, Heo.; Eun-Kyoung, Ko.; Ki-Yeon, Kim.; Young-Jo, Kim.; Hyang-Jin, Yoon.; Sung-Hwan, Wee.; Yong Ho, Park. and Jin San Moon (2015): Antibiotic Resistance and Virulence Potentials of Shiga Toxin-Producing *Escherichia coli* Isolates from Raw Meats of Slaughterhouses and Retail Markets in Korea J. Microbiol. Biotechnol. 25(9): 1460–1466.

ICMSF (2002): Microorganisms in Food 7: Microbiological testing in food safety management. Kluwer Academic/Plenum Publishers, New York.

Johnson, K.E.; Thorpe, C.M. and Sears, C.L. (2006): The emerging clinical importance of non-O157 Shiga toxin-producing *Escherichia coli*. Clin Infect Dis. 43(12): 1587-95.

Kesava, Naidu. G.; Rajendra, Goud N.; Gaddad S.M. and Shivannavar, C.T. (2011): Detection of shiga toxin genes (stx1&stx2) and molecular characterization of shiga toxigenic *Escherichia coli* isolated from diverse sources in Gulbarga region, India. Pharmacophore. 2 (5): 253-265.

Krishnan, C.; Fitzgerald, V.; Dakin, S. and Behme, R. (1987): Laboratory investigation of outbreak of HC caused by *E. coli* O157:H7. J. Clin. Microbiol., 25: 1043-1047.

Liao, Y.T.; Miller, M.F.; Loneragan, G.H.; Brooks, J.C.; Echeverry, A. and Brashears, M.M. (2014): Non-O157 Shiga toxin-producing *Escherichia coli* in U. S. retail ground beef. J Food Prot. 77(7): 1188-92.

March, S.B. and Ratnam, S. (1989): Latex agglutination test for detection of *E. coli* serotype O157. J. Clin. Microbiol., 27(7): 1675-1677.

Meng, J.; LeJeune, J.T.; Zhao, T. and Doyle, M.P. (2013): Enterohemorrhagic *Escherichia coli*. Ch 12 In: Doyle MP, Beuchat LR (eds) Food microbiology: Fundamentals and frontiers. 4th ed, ASM Press, Washington D.C. 287–309.

Miko, A.; Pries, K.; Haby, S.; Steege, K.; Albrecht, N.; Krause, G. and Beutin, L. (2009): Assessment of Shiga toxin-producing *Escherichia coli* isolates from wildlife meat as potential pathogens for humans. Applied and Environmental Microbiology 75: 6462–6470.

Mohammed, M.A.; Sallam, K.I.; Eldaly, E.A.; Ahdy, A.M. and Tamura, T. (2013): Occurrence, serotypes and virulence genes of non-O157 Shiga toxinproducing Escherichia coli in fresh beef, ground beef, and beef burger. Food Control 37:182-187.

Momtaz, H. and Jamshidi, A. (2012): Shiga toxin producing *Escherichia coli* isolated from chicken meat in Iran Serogroups, virulence factors, and antimicrobial resistance properties. Poultry Science. 92(5): 1305-1313.

Momtaz, H.; Dehkordi, F.S.; Rahimi, E.; Ezadi, H. and Arab, R. (2013): Incidence of Shiga toxin-producing *Escherichia coli* serogroups in ruminant's meat. Meat Science. 95(2): 381-388.

Momtaz, H.; Rahimi, E. and Moshkelani, S. (2012): Molecular detection of antimicrobial resistance genes in *E. coli* isolated from slaughtered commercial chickens in Iran. Veterinarni Medicina, 57 (4): 193–197.

Mora, A.; Blanco, M.; Blanco, J.E.; Dahbi, G.; Lopez, C.; Jusl, P.; Alonso, M.P.; Echeita, A.; Berardez, M.I.; Gonzaiez, E.E. and Blanco, J. (2007): Serotypes, virulence genes and intimin types of shiga toxin (Verocytotoxin)–producing *Escherichia coli* isolate from minced beef in lugo (Spain) from 1995 through 2003. BMC Microbiology 7: 13.

Nataro, J.P. and Kaper, J.B. (1998): Diarrheagenic *Escherichia coli*. Clin. Microbiol. Rev. 11: 132-201.

Nguyen, Do. Phuc.; Nguyen, Thi. Anh. Dao.; Thi, Hien. Le.; Nguyen, Minh. Doan. Tran.; Thanh, Phong. Ngo.; Van, Chinh. Dang.; Takao, Kawai.; Masashi, Kanki.; Ryuji, Kawahara.; Michio, Jinnai.; Shinya, Yonogi.; Yuji, Hirai.; Yoshimasa,Yamamoto. and Yuko, Kumeda. (2016): Dissemination of Extended-Spectrum -Lactamase- and AmpC -Lactamase-Producing *Escherichia coli* within the Food Distribution System of Ho Chi Minh City, Vietnam. BioMed Research International 12(8): 719-725.

Panahee, M. and Pourtaghi, H. (2016): Virulence gene profiles of Shiga-toxin producing *Escherichia coli* isolates from retail raw meat in Iran. Bulg. J. Vet. Med. (online first). 1311-1477.

Paton, A.W. and Paton, J.C. (2002): Direct detection and characterization of Shiga toxigenic *Escherichia coli* by multiplex PCR for stx1, stx2, eae, ehxA, and saa. J. Clin. Microbiol. 40: 271-274.

Paton, J.C. and Paton, A.W. (1998): Pathogenesis and diagnosis of Shiga toxin-producing *Escherichia coli* infections. Clin. Microbiol. Rev. 11: 450-479.

Patricia, Llorente.; Laura, Barnech.; Kinue, Irino. María.; Valeria, Rumi. and Adriana, Bentancor. (2014): Characterization of Shiga Toxin-Producing *Escherichia coli* Isolated from Ground Beef Collected in Different Socioeconomic Strata Markets in Buenos Aires, Argentina. Sao Paulo, SP, Brazil. Instituto Adolfo Lutz, 01246-000.

Perelle, S.; Dilasser, F.; Malorny, B.; Grout, J.; Hoorfar, J. and Fach, P. (2004): Comparison of PCR, ELISA and light cycler real time PCR assays for detecting *Salmonella spp*. in milk and meat samples Molecular and Cellular Probes, 18 (6): 409-420.

Pradel, N.V.; Livrelli, C.; De Champs, J.B.; Palcox, A.; Reynaud, F.; Scheutz, J.; Sirot, B.; Joly, and C. Forestier. (2000): Prevalence and characterization of Shiga toxin-producing *Escherichia coli* isolated from cattle, food, and children during a one-year prospective study in France. J. Clin. Microbiol. 38: 1023–1031.

Quinn, P.J.; Carter, M.E.; Markey, B.K. and Carter, G.R. (1994): Clinical Veterinary Microbiology. Mosby Wolfe, 14, p: 4251-4255.

Scheutz, F. and Strockbine, N.A. (2005): Genus I. Escherichia. In: Brenner, D.J., *et al.* (Eds.) The Protebacteria Part B The Gammaproteobacteria. Springer. pp. 607-623.

Sethulekshmi, C.; Latha, C. and Sunil, B. (2016): Occurrence of Enterohaemorrhagic *E. coli* in raw meat samples in Kerala. Int. J. Adv. Res. Biol. Sci. 3(1): 220–222.

Soliman, Z.I. and El-Tabiy, A.A. (2006): A study on occurrence of *Escherichia coli* in some beef products with special reference to *E. coli* O157: H7. Assiut Vet. Med. J., 52 (110): 75–87.

Verweyen, H.; Karch, H.; Brandis, M. and Zimmerhackl, L. (2000): Enterohemorrhagic *Escherichia coli* infections: following transmission routes. Pediatr Nephrol, 14: 73. https://doi.org/ 10.1007/s004670050018.

Wenting, J.U.; Jinling, Shen.; Yi Li.; Magaly, A. Toro.; Shaohua ,Zhao.; Sherry, Ayers.; Mohamed, Badaoui. Najjar. and Jianghong, Meng. (2012): Non-O157 Shiga toxin-producing *Escherichia coli* in retail ground beef and pork in the Washington D.C. area. Food Microbiology 32(2):371–377.

Zaki, E. and Mahrouk, A.M. (2005): Rapid detection of Enterotoxigenic *E. coli* recovered from buffalo meat products using PCR. Assiut Vet. Med. J. 51(104): 35-49.

Zende, R.J.; Chavhan, D.M.; Suryawanshi, P.R.; Rai, AK. and Vaidya, V.M. (2013): PCR detection and serotyping of enterotoxigenic and shigatoxigenic *Escherichia coli* isolates obtained from chicken meat in Mumbai, India. Veterinary World, 6(10): 770-773.

Zhao, C.; Ge, B.; Villena, de. J.; Sudler, R.; Yeh, E.; Zhao, S.; White, D.G.; Wagner; D. and Meng, J. (2001): Prevalence of *Campylobacter spp., Escherichia coli*, and *Salmonella* serovars in retail chicken, turkey, pork, and beef from the greater Washington, D.C., area. Appl. Environ. Microbiol. 67, 5431-5436.

معدل حدوث ميكروب الأيشريكية القولونية المنتجة لسموم الشيجا فى اللحوم الحمراء واللحوم المفرومة ولحوم الدواجن وإسهال الأطفال

محمد كرمى ، سوزان إسماعيل

E-mail: karmy99@yahoo.com Assiut Univers ity web-site: www.aun.edu.eg

أجريت هذه الدراسة على عدد ٢٠٠ عينة من اللحوم الحمراء واللحوم المفرومة ولحوم الدواجن وإسهال الأطفال و تتكون من ٥٠ عينة من كل منها تم تجميعها من المحال المختلفة لبيع اللحوم فى محافظة أسوان فى عام ٢٠١٨. تم فحص هذه العينات بكتريولوجيا وكيميائيا و سيرولوجيا وجينيا للكشف على ميكروب الأيشريكية القولونية المنتجة لسموم الشيجا والجينات الخاصة بها وهم *stx*1, *stx*2 and *eae*A. وقد أوضحت النتائج أن معدل حدوث ميكروب الأيشريكية القولونية فى اللحوم الحمراء واللحوم المفرومة ولحوم الدواجن وإسهال الأطفال هو ٢٢% و ١٨% و ١٢% و ١٢% على التوالى بينما معدل حدوث جينات الضراوة كالتالى: جين الشيجاتوكسين ١ هو ٣٣-٧٧% و جين الشيجاتوكسين ٢ هو ٣٦-٨٣% وجين الأنتيمين أ هو ١٦-٣٦%. نستخلص من هذه الدراسة أن ميكروب الأيشريكية القولونية المنتجة لسموم الشيجا موجود فى اللحوم الحمراء واللحوم المفرومة ولحوم الدواجن وينتقل إلى الأطفال من خلال الأطعمة الملوثة. تنصح الدراسة بضرورة التأكد من الطبخ الجيد و الحرارة الكافية للحوم قبل تناولها حتى يمكن تجنب العدوى والتسمم الغذائى بهذا الميكروب الخطير.